Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/007273

International filing date: 03 March 2005 (03.03.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/550,261
Filing date: 03 March 2004 (03.03.2004)

Date of receipt at the International Bureau: 18 April 2005 (18.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

1305367

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APPLICATION NUMBER: *60/550,261*
FILING DATE: *March 03, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/07273*

Certified by

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PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EV 313 811 368 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Thomas E.	Daley	San Mateo, CA

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHODS FOR INHIBITING ALCOHOL TOXICITY

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 23,639

OR

☐ Firm or Individual Name	Bingham McCutchen LLP				
Address	Three Embarcadero Center				
Address	Suite 1800				
City	San Francisco	State	CA	ZIP	94111-4067
Country	CA	Telephone	(650) 849-4950	Fax	(650) 849-4800

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 19 ☐ CD(s), Number _____

☒ Drawing(s) *Number of Sheets* 1 ☒ Other (specify) Return Postcard

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

FILING FEE AMOUNT ($)

☒ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-2518 — 80.00

☐ Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE 

Date March 3, 2004

REGISTRATION NO. (*if appropriate*) 34,202

TYPED or PRINTED NAME Antoinette F. Konskil

Docket Number: TD 3001.00

TELEPHONE (650) 849-4950

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	March 3, 2004
First Named Inventor	Thomas E. Daley
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Attorney Docket No.	TD 3001.00

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 50-2518

Deposit Account Name: Bingham McCutchen LLP

The Director is authorized to: *(check all that apply)*

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☐ Charge any additional fee(s) during the pendency of this application

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		-20 ** =	0	X		=	0
Independent Claims		-3 ** =	0	X		=	0
Multiple Dependent				X		=	0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
				Other fee (specify) _____	

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) 0

SUBMITTED BY				Complete *(if applicable)*	
Name (Print/Type)	Antoinette F. Konski	*Registration No. (Attorney/Agent)*	34,202	*Telephone*	(650) 849-4950
Signature				*Date*	March 3, 2004

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METHODS FOR INHIBITING ALCOHOL TOXICITY

BACKGROUND OF THE INVENTION

Throughout and within this disclosure, various publications, patents, published patent applications and references are identified by first author and date, within parentheses, or alternatively, by a patent number, a publication number or by a web address. If the complete bibliographic citation is not provided after the publication or reference, it is at the end of the specification, immediately preceding the claims. The disclosures of all publications, references and information provided at the web addresses are hereby incorporated by reference into this disclosure to more fully describe the state of the art to which this invention pertains.

Ethanol-based beverages have been used both recreationally and medicinally in human cultures for the length of recorded history. However, a significant portion of the population produces a defective version of aldehyde dehydrogenase subtype 2 (ALDH2), the mitochondrial liver enzyme necessary to metabolize alcohols safely. This results in a 40-90% reduction in the rate of acetaldehyde oxidation. For humans with the normal enzyme, long-term use of alcohol in high doses leads to various health disorders, including liver cirrhosis and hepatocellular carcinoma. For humans with mutant versions of the enzyme, these disorders can result from ingestion of alcohol at relatively low doses over a shorter period of time (Ohira 1996, Takeshita 2000). The experience of alcohol consumption is marked by facial flushing, accelerated heart rate, and a subjective sense of sickness rather than the euphoria and/or relaxation that typically accompanies alcohol consumption (Ward 1994).

Humans with ALDH2 deficiencies are also more susceptible to cancers of the gastrointestinal tract, including the mouth, stomach and esophagous (Nomura 2000). These currently are among the least treatable of cancers, making this a critical health problem and the cause of thousands of deaths per year.

Alcohols are metabolized through a two-step pathway. Free alcohol in the bloodstream is oxidized by a variant of alcohol dehydrogenase (ADH), which results in the production of acetaldehyde. Acetaldehyde is then broken down to acetic acid in water.

The acetaldehyde intermediate is the molecule responsible for the deleterious effects of alcohol consumption among both healthy subjects and those with mutant forms of the ALDH2 enzyme. This molecule is well known as a toxin. It has been found to have cytogenetic effects (Bohlke 1983) and to cause oxidative stress in hepatocytes (Eriksson 2001).

Previous approaches to the problem have focused on the ALDH2 enzyme itself. This enzyme is tetrameric and, at 216 kD, quite large (Sladek 2002). It also requires post-translational modification via chaperone molecules to combine the translated monomers into its tetrameric form. For these reasons it is difficult to synthesize in commercial quantity. Some practitioners have suggested synthesis in plants, but this has not been successfully reduced to practice.

If it could be synthesized, administration of synthesized wildtype ALDH 2 to human subjects presents additional difficulties. The molecule's weight prevents it from transferring across epithelial tissue or transdermally as small organic molecules might. The half-life in blood is short, requiring the administration of large quantities. Furthermore, the molecule is quickly degraded in the gastrointestinal tract. Thus, traditional oral, pulmonary, or transdermal methods of drug delivery are ineffective.

DESCRIPTION OF THE INVENTION

This invention provides methods and examples of compounds for inhibiting the oxidization of ethanol to acetaldehyde in hepatocytes. In one aspect, a molecule is administered or delivered to a cell, and the molecule competitively binds to alcohol dehydrogenase (ADH) enzyme. In another aspect, the compound is administered or delivered as a pharmaceutical composition suitable for oral administration. An example of a compound is fomepizole, such as 4-methyl or 4-ethylpyrazole. In one aspect, the compound for use in the method of this invention

is a hydrochloride salt of 4-methylpyrazole. It is understood that 4-substituted pyrazoles, and in one embodiment, 4-ethylpyrazole, will act through the same mechanism, and that hydrochloride salts of 4-substituted pyrazoles comprise examples of such compounds. The compound may be used alone or in combination with other therapeutics or alternative therapies. Alternative therapies include, but are not limited to the administration of an effective amount of kudzu root derivatives and/or soy products.

Also provided by this invention is a method for preventing, treating or ameliorating symptoms associated with dysregulation of the metabolism of alcohol in subjects, e.g., subjects who cannot metabolize alcohol due to non-functional ALDH2 enzyme activity. Examples of symptoms include, but are not limited to flushing and accelerated heart rate when ethanol is orally consumed. The method requires administration of an effective amount of a compound that inhibits the oxidation of ethanol to acetaldehyde in hepatocytes. The compound may be used alone or in combination with other therapeutics or alternative therapies. Alternative therapies include, but are not limited to the administration of an effective amount of kudzu root derivatives and/or soy products.

A further aspect of this invention is the preparation of a medicament for use in preventing or treating a pathology or symptoms caused by a variation in structure of the liver mitochondrial enzyme ALDH2, that leads to non-functional enzyme activity in the host or subject.

This invention also provides a method for identifying potential therapeutic compounds by contacting a ADH enzyme or a cell containing ADH enzyme with a candidate therapeutic agent or prodrug which competitively binds with ADH in the medium or cell. In one aspect, these cells are human hepatocytes. In another aspect, they are of hematological or cardiac origin.

After the cell is contacted *in vitro* and/or *in vivo* with the candidate compound or agent, the cell is assayed for efficacy of the agent by noting the concentration of acetaldehyde and/or the presence or absence of NADH. Further provided by this invention is a kit for testing potential therapeutics containing at least one reagent described herein and instructions for use.

BRIEF DESCRIPTION OF THE FIGURE

The Figure depicts in schematic form the metabolic pathway primarily responsible for ethanol metabolism.

MODE(S) FOR CARRYING OUT THE INVENTION

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "MOLECULAR CLONING: A LABORATORY MANUAL" Second Edition (Sambrook et al., 1989); "OLIGONUCLEOTIDE SYNTHESIS" (M.J. Gait, ed., 1984); "ANIMAL CELL CULTURE" (R.I. Freshney, ed., 1987); the series "METHODS IN ENZYMOLOGY" (Academic Press, Inc.); "HANDBOOK OF EXPERIMENTAL IMMUNOLOGY" (D.M. Weir & C.C. Blackwell, eds.); "GENE TRANSFER VECTORS FOR MAMMALIAN CELLS" (J.M. Miller & M.P. Calos, eds., 1987); "CURRENT PROTOCOLS IN MOLECULAR BIOLOGY" (F.M. Ausubel et al., eds., 1987, and periodic updates); "PCR: THE POLYMERASE CHAIN REACTION" (Mullis et al., eds., 1994); "CURRENT PROTOCOLS IN IMMUNOLOGY" (J.E. Coligan et al., eds., 1991); and J. March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS AND STRUCTURE, 4th edition (John Wiley & Sons, NY (1992)).

Definitions

As used herein, certain terms may have the following defined meanings.

As used in the specification and claims, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. Similarly, use of "a compound" for treatment or preparation of medicaments as described herein contemplates using one or more compounds of this invention for such treatment or preparation unless the context clearly dictates otherwise.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

As used herein, the term "analog" is intended to mean a structural derivative of a compound that differs from it by at least one element. The term "derivative" is intended to mean a compound derived or obtained by another and containing the essential elements of the parent substance.

The term "alkyl" refers to and covers any and all groups which are known as normal alkyl, branched-chain alkyl and cycloalkyl. As used herein, "alkyl" is intended to include both branched, straight-chain, substituted or unsubstituted saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, and s-pentyl.

Lower alkyl means the above-defined broad definition of alkyl groups having 1 to 6 carbons in case of normal lower alkyl, and as applicable 3 to 6 carbons for lower branch chained and cycloalkyl groups. Lower alkenyl is defined similarly having 2 to 6 carbons for normal lower alkenyl groups, and 3 to 6 carbons for branch chained and cyclo- lower alkenyl groups. Lower alkynyl is also defined similarly, having 2 to 6 carbons for normal lower allynyl groups, and 4 to 6 carbons for branch chained lower alkynyl groups.

Some of the compounds of the present invention may have trans and cis (E and *Z*) isomers. In addition, the compounds of the present invention may contain one or more chiral centers and therefore may exist in enantiomeric and diasteromeric forms. Still further oxi and related compounds of the present

invention may exist in syn and anti isomeric forms. The scope of the present invention is intended to cover all such isomers per se, as well as mixtures of cis and trans isomers, mixtures of syn and anti isomers, mixtures of diastereomers and racemic mixtures of enantiomers (optical isomers) as well. In the present application, when no specific mention is made of the configuration (cis, trans, syn, anti, R or S) of a compound (or of an asymmetric carbon) then a mixture of such isomers, or either one of the isomers is intended. In a similar vein, when in the chemical structural formulas of this application a straight line representing a valence bond is drawn to an as etric carbon, then isomers of both R and S configuration, as well as their mixtures are intended. Defined stereochemistry about an asymmetric carbon is indicated in the formulas (where applicable) by a solid triangle showing beta configuration, or by a hashed line showing alpha configuration.

All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

A "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

A "composition" is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include

stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975)).

An "effective amount" is an amount sufficient to effect beneficial or desired results. For example, a therapeutic amount is one that achieves the desired therapeutic effect. This amount may be the same or different from a prophylatically effective amount, which is an amount necessary to prevent onset of disease or disease symptoms. An effective amount can be administered in one or more administrations, applications or dosages.

A "control" is an alternative subject or sample used in an experiment for comparison purpose. A control can be "positive" or "negative". For example, where the purpose of the experiment is to determine a correlation of an altered expression level of a enzyme or product with a particular disease, condition or pathology, it is generally preferable to use a positive control (a subject or a sample from a subject, carrying such alteration and exhibiting symptoms characteristic of that disease or condition), and a negative control (a subject or a sample from a subject lacking the altered expression and clinical symptoms of that disease).

Description of the Invention

Practitioners in the art will recognize that up-regulation of the ALDH2 activity, like up-regulation of most enzymes, is considered to be difficult to undertake. To overcome this and other limitations of the prior art, the invention provides a method for treating or ameliorating the symptoms in a subject associated with dysregulation of the ALDH2 enzyme in a subject. In one aspect, the method is administered after ingestion of an alcohol-containing product.

In one aspect, the method comprises administering or delivering to the subject in need thereof an effective amount of a substituted pyrazole or its pharmaceutically acceptable salt. Examples of suitable substituted pyrazole compounds include, but are not limited to 4-alkylpyrazole, e.g., 4-methyl or 4-ethylpyrazole.

In one aspect, the compound for use in the method of this invention is a hydrochloride salt of 4-methylpyrazole. It is understood that 4-substituted

pyrazoles, and in one embodiment, 4-ethylpyrazole, will act through the same mechanism, and that hydrochloride salts of 4-substituted pyrazoles comprise examples of such compounds.

These compounds are commercially available from Sigma Aldrich, Alfa Aezar and Fluka or can be synthesized easily in commercially viable quantities of pharmaceutical grade.

The compounds can be delivered alone or in combination with other therapies or active agents. The compounds are delivered by any means known in the art, but are preferably delivered by a method suitable for the prevention or treatment of a chronic condition such as orally or transdermally. An effective amount is delivered, which in one aspect is sufficient to maintain a serum level of from 0.1 to 10 mg/kg, or alternatively, less than 10 mg/kg or yet further, about 5 mg/kg of subject body weight.

Examples of symptoms associated with the dysregulation of ALDH2 enzyme in a host cell or subject include, flushing, gastrointestinal or hepatocellular cancer, metabolic acidosis, renal failure, hypocalcemia, oxaluria, damage to the nervous system and cardiovascular instability.

There is support in the literature for the hypothesis that inhibition of the oxidizing activity of the ADH enzyme, of which acetaldehyde is the resulting metabolite, can reduce levels of blood acetaldehyde (Sarkola 2001, Sarkola 2002). This is reported to ameliorate the deleterious effects of alcohol consumption in subjects with ALDH2 deficiency.

This mechanism of action also is accepted as being responsible for acute conditions such as ethylene glycol poisoning and methanol poisoning (Brent 1999, Brent 2001). In these pathologies, ethylene glycol or methanol are metabolized to oxalates and glycolates that lead to metabolic acidosis, renal failure, hypocalcemia, oxaluria, damage to the nervous system and cardiovascular instability. The commercial composition fomepizole, a medicament comprising the 4-methyl form of pyrazole ("4-MP"), is currently approved and administered for this condition. However, that composition requires injection under the supervision of a doctor in relatively large doses over a short period of time.

Notably, fomepizole is not used in cases of ethanol poisoning, and in fact is narrowly indicated. The current invention expands the use of this molecule, and further includes methods of administration that are more appropriate for treating or preventing a chronic condition. Current methods of administration and dosing known in the art are inappropriate and/or inconvenient for the treatment of a chronic condition associated with ALDH2 deficiency. The current invention therefore overcomes deficiencies of prior methods. The oral route of administration also can be self-administered by the subject.

Fomepizole's injectable administration has advantages in an emergency care setting, but is inappropriate in the treatment of ALDH2 deficiency, a chronic condition requiring daily care that the patient can administer to him or herself. Non-injectable administration will also yield greater efficacy in the contemplated indication, as the patient is much more likely to be able to stay within the therapeutic window of the pyrazole drug when self-administering via an oral or similar route.

Furthermore, it is undesirable that the rate of ethanol oxidation be too low, with the consequence being overly-lengthy periods of time during which the subject is under the influence of ethanol.

Current dosing regimens for 4-MP in the acute care context are predicated on the need to reduce the end metabolite concentrations to very low levels in order to prevent severe damage to multiple organ systems. Although elevated acetaldehyde concentrations carry significant health risks, these risks are not as acute nor as imminent as in the contexts for which fomepizole is administered. Persistence of the substrate in the subject blood stream for time periods measured in days — as is the case with currently practiced dosing of fomepizole — is highly undesirable for subjects with ALDH2 deficiency or dysregulation who wish to consume alcohol, as they typically wish the effects of elevated blood alcohol levels to be temporary, lasting from minutes to several hours.

Certain practitioners have demonstrated that the mechanism of action of 4-methylpyrazole can be utilized to reduce blood acetaldehyde concentrations and prevent the flushing responses and cardiac hypertachia found in ALDH2-deficient patients. However, these studies used doses which suffer from two critical

problems that would prohibit use in the application of the current invention. The first that ADH is over-inhibited resulting in blood ethanol levels that peaked much higher than untreated patients and an undesirably high AUC (area under the curve) for blood ethanol levels. While some elevation in AUC is inevitable in this indication, the metabolization of ethanol at the 4-MP doses described in the literature is too slow to allow safe recreational use of ethanol. Blood ethanol concentrations at these higher doses of 4-MP remain at a 2-5 mM level after 5 hours; ideally ethanol concentrations would be no higher than 1 mM after that period of time.

Furthermore, the dosages taught in the literature have an undesirable side effect profile with chronic use. While appropriate in the acute care context, preclinical data in mice indicate the danger of up to 30% shrinkage of the testicular mass after several weeks of use. The current invention contemplates doses low enough to avoid this effect.

In Vitro Use and Drug Assay

This invention also provides a method for identifying agents which have therapeutic potential for the prevention, treatment or amelioration of symptoms associated with ALDH2 deficiency. The agent is considered a potential therapeutic agent if ADH enzyme inhibition is noted in vitro using methods known in the art. To practice the assay, an effective amount of the candidate drug or agent is contacted with a cell expressing ADH enzyme.

Suitable cells for use in the assay include, but are not limited to cells that endogenously produce ADH, e.g., hepatocytes. Alternatively, the cells can be recombinantly prepared to express ADH. The recombinant cells can be procaryotic (bacterial such as *E. coli*) or eucaryotic. Alternatively, the recombinant cells can be mammalian or non-mammalian cells, e.g., mouse cells, rat cells, human cells or fungi (e.g., yeast).

Human alcohol dehydrogenases are available through commercial sources, including Sigma-Aldrich or alternatively, can be cloned using gene sequences available in the literature e.g., the GenBank database. Various pyrazole derivatives, including 4-methylpyrazole, are also commercially available.

Inhibition constants can be determined by initial velocity studies in sodium phosphate (46 mM) and 0.25 mM EDTA buffer, pH 7.0, at 25° C. The change in absorbance can be measured at 340 nm, which captures the formation or utilization of NADH. The coenzyme concentration can he held constant, and the concentration of the ADH. The concentrations of the inhibitor can ha varied over a 3-fold range, with duplicates utilizing various assay conditions. The inhibition data can be fitted to the Michelis-Menten equations using computer software known in the art.

The potentially therapeutic agent identified by the method of this invention can also be a prodrug of a pyrazine derivative. As used herein, a "prodrug" is a precursor or derivative form of a pharmaceutically active agent or substance that is metabolized in vivo to the biologically or chemically active substance.

It may be desirable to perform the assay using two cell types, the first being a control cell in which the enzyme is expressed at normal levels. The second cell type is the test cell, in which the enzyme is expressed at an altered, higher or lower level. In another embodiment, a third target cell is used as a control because it receives an effective amount of a compound, such as, for example, a pyrazine derivative, which have been shown to be therapeutic. This embodiment is particularly useful to screen for new agents.

In Vivo Use

The *in vitro* assays are confirmed in animal models to determine *in vivo* efficacy. Alternatively, the compounds are administered to a subject such as a human patient for their therapeutic benefit. 4-methylpyrazole can be obtained from commercially available sources. The lyophilized form of 4-MP can be administered in encapsulated form. Determination of efficacy of potential or new compounds or therapies can be determined by monitoring blood acetaldehyde levels, heart rate (which is typically elevated in the presence of elevated blood acetaldehyde), and blood ethanol concentrations.

Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration for treatment or for

prophylactic use are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

The agents and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. In addition to a compound of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds.

In general, a suitable dose for each of the above-named compounds, is in the range of less than 20 mg per kilogram body weight of the recipient per day, in one aspect the range of about 1 to about 10 mg per kilogram body weight per day and in another aspect in the range of about 1 to about 5 mg per kilogram body weight per day. Unless otherwise indicated, all weights of active ingredient are calculated as the parent compound of the formula of the present invention for salts or esters thereof, the weights would be increased proportionately. It will be appreciated that appropriate dosages of the compounds and compositions of the invention can vary from patient to patient.

Ideally, the compounds should be administered to achieve peak concentrations of the active compound within hepatocytes.

While it is possible for the compound to be administered alone, it is preferable to present it as a pharmaceutical formulation comprising at least one active ingredient, as defined above, together with one or more pharmaceutically acceptable carriers and optionally other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing

a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions for topical administration according to the present invention may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The compound preferably

present in such formulation in a concentration of about 0.5 to about 20%, advantageously about 0.5 to about 10% particularly about 1.5% w/w.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable of oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

Compounds and compositions of the formula of the present invention may also be presented for the use in the form of veterinary formulations, which may be prepared, for example, by methods that are conventional in the art.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

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CLAIMS

We claim:

1. A method of preventing or treating a deficiency or mutation associated with a mutated ALDH enzyme protein in a subject or alternatively, preventing, treating or ameliorating the symptoms associate with ALDH dysregulation in a subject, such method comprising administering an effective amount of a pyrazole derivative that inhibits the ethanol-oxidizing activity of the Alcohol Dehydrogenase (ADH) enzyme.

2. The method of claim 1, wherein the compound is administered orally.

3. The method of claim 1, wherein the compound is administered transdermally.

4. The method of claim 1, wherein the compound is administered via a pulmonary route.

5. The method of claim 1, wherein the compound is a pyrazole derivate.

6. The method of claim 5, wherein the compound is 4-alkylpyrazole.

7. The method of claim 5, wherein the compound is 4-methylpyrazole.

8. The method of claim 5, wherein the compound is 4-ethylpyrazole.

9. The method of claim 5, wherein the compound has been modified to utilize an active transport mechanism to increase the bioavailability.

10. The method of claim 6, wherein the compound is a hydrochloride salt.

11. The method of claim 7, wherein the compound is a hydrochloride salt.

12. The method of claim 8, wherein the compound is a hydrochloride salt.

13. The method of claim 9, wherein the compound is a hydrochloride salt.

14. The method of claim 7, wherein the effective amount comprises less than 10 mg/kg body weight of the subject.

ABSTRACT

Methods and examples of compounds for inhibiting the oxidization of ethanol to acetaldehyde in hepatocytes are provided by this invention. Also provided is a method for preventing, treating or ameliorating symptoms associated with dysregulation of the metabolism of alcohol in subjects, e.g., subjects who cannot metabolize alcohol due to non-functional ALDH2 enzyme activity. Examples of symptoms include, but are not limited to flushing and accelerated heart rate when ethanol is orally consumed. The method requires administration of an effective amount of a compound that inhibits the oxidation of ethanol to acetaldehyde in hepatocytes. The compound may be used alone or in combination with other therapeutics or alternative therapies. Alternative therapies include, but are not limited to the administration of an effective amount of kudzu root derivatives and/or soy products.

The Figure